CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society

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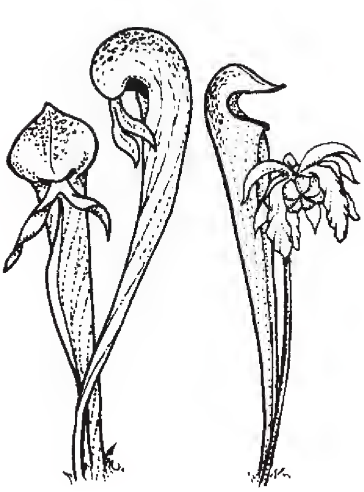

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Front Cover: *Pinguicula macroceras* at Site 5, in California. Photo by Barry Rice. Article on page 44.

Back Cover: Barry Rice wrassling with *Utricularia foliosa* in western Florida. Photo by Elizabeth Salvia.

Carnivorous Plant Newsletter is dedicated to spreading knowledge and news related to carnivorous plants. Reader contributions are essential for this mission to be successful. Do not hesitate to contact the editors with information about your plants, conservation projects, field trips, or noteworthy events. Advertisers should contact the editors. Views expressed in this publication are those of the authors, not the editorial staff.

All correspondence regarding dues, address changes and missing issues should be sent to the Membership Coordinator at the ICPS. Do not send such correspondence to the editors. Checks for subscriptions should be made to the ICPS in US funds. Dues for 2011 are $35 for the first year of membership; renewals are $30 per year.

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From the President

Hello, my name is Mike Baldwin and I'm the president of ICPS.

It's been a busy year for the ICPS, and I wanted to take a moment to share a few of the larger activities that the ICPS has been up to recently.

- *ICPS Conference 2010 and 2012*: ICPS Conference 2010 in Leiden was a great success. The dust did not have time to settle before talks about the ICPS 2012 started to germinate. The New England Carnivorous Plant Society was selected to host the ICPS Conference 2012 to be held August 11–13, 2012, in Seekonk, Massachusetts with field trips following for several days afterward. To receive conference announcements, please send an email to Emily Troiano, NECPS Vice President, at emily.troiano@gmail.com.
- *Rare Nepenthes Conservation Project*: Previously mentioned in the CPN, one of the things we've been very excited about is our partnership with The Ark of Life, and the Hortus Botanicus Leiden in establishing the *Rare Nepenthes Collection*. Thank you to the folks who have contributed time, money, and plant material! If you have a moment, please check out the website (www.arkoflife.net).
- *Social Media*: The ICPS is 39 years old (WOW!). While we've had the CP e-mail Listserv, Website, and Forum for a few years now, thanks to the help of awesome volunteers, the ICPS has stepped firmly into the 21st Century by entering into the world of social media. If you have not seen them yet, the ICPS now has a Facebook page, a Flickr photo sharing group, and the ICPStv channel on YouTube that allows members and people in the extended CP family to connect and share their CP experiences! If you go to our website (www.carnivorousplants.org), you can follow the web links to each.

As our name states, we are an international organization with about 1400 members around the globe. Over the past year, members of the board have had some interesting discussions about what the word "international" in our name means. I would like to hear from you – if you have a moment, send me an email about the word "International" and what you think that means for the ICPS.

We have some great volunteers who help the ICPS on a regular basis, and we're on the lookout for more! If you want to help out on a project, let me know. In the next year, we will have some projects and activities that will need your help.

Lastly, I'd like to thank all of you who have donated or contributed to the various ICPS conservation projects or projects closer to home. Your contributions of time and money are greatly appreciated, and make a difference. If you'd like to make a donation, or know of a conservation project, let us know.

I look forward to hearing from you!

Mike Baldwin
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Flower studies do not support subspecies within *Pinguicula macroceras*

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Keywords: Field studies: *Pinguicula macroceras,* California.

Abstract

How much do the flowers of *Pinguicula* vary in size from site to site? To explore this and related questions, flowers of *Pinguicula macroceras* from different Californian colonies were studied. It was found that different colonies, even if separated by only small distances, can have significantly different typical flower sizes. These differences are even greater than the differences that were used to define subspecies within *Pinguicula macroceras*. As such, there is inadequate evidence to support subspecific divisions within *P. macroceras*.

Introduction

As shown in Figure 1, *Pinguicula macroceras* Link ranges from the western USA, north to Canada, and west to Russia and Japan (Casper 1966; Schlauer 2002; Schnell 2002). The range is highly uncertain as can be seen by comparing the maps in Casper (1966) and Schnell (2002), and is a topic worthy of further study. The species is often divided into two subspecies: *Pinguicula macroceras* Link subsp. *macroceras* and *Pinguicula macroceras* subsp. *nortensis* J. Steiger & J.H. Rondeau (Rondeau & Steiger 1997). The details of the differences between the two subspecies are noted in the discussion, but briefly they are (1) the spur and corolla length, (2) the shape and degree of overlap in the lower corolla lobes, and (3) the shape of the tips of the calyx lobes.

Recently, a population of *Pinguicula* in the Castle Crags district of north-central California was investigated for the first time (Rice *et al.* 2008; hereafter RYM2008). This location represented a range extension for *Pinguicula macroceras*, and it was not clear whether the plants should be classified as *Pinguicula macroceras* subsp. *nortensis* or *Pinguicula macroceras* subsp. *macroceras*. While the Castle Crags plants were geographically closest to the range of *Pinguicula macroceras* subsp. *nortensis*, the flowers were larger (24-38 mm) than the size described for *Pinguicula macroceras* subsp. *nortensis* (13-21 mm). This discovery encouraged further study, especially of the variability of flower sizes within the species.

Within its range, *Pinguicula macroceras* subsp. *nortensis* occurs in pocket populations that are separated by distances both large and small. The overall character of the plants (for example, flower sizes) may be different from site to site. However, taken as a whole, the variation of the plants still falls within the definition of the species, *Pinguicula macroceras*. Meanwhile, how important are the floral differences that are used to distinguish the two subspecies of *Pinguicula macroceras*? While the variation in site-to-site flower sizes may be due to both environmental and genetic differences, if the site-to-site differences in *Pinguicula macroceras* subsp. *nortensis* plants is greater than the differences used to define the two subspecies, then the subspecies separation is not merited. The current study, therefore, explores this variation within *Pinguicula macroceras* in California.

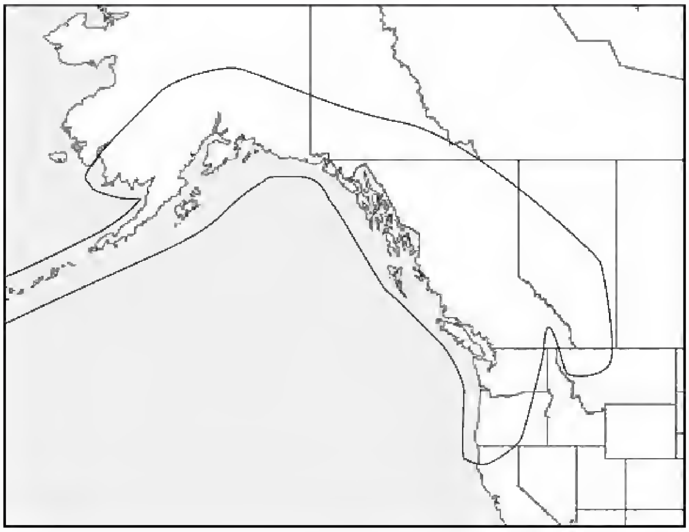

Figure 1: The range of *Pinguicula macroceras* in North America. The boundaries of the range are uncertain, especially along the western coast of Alaska. Within the range indicated, sites are often separated by great distances.

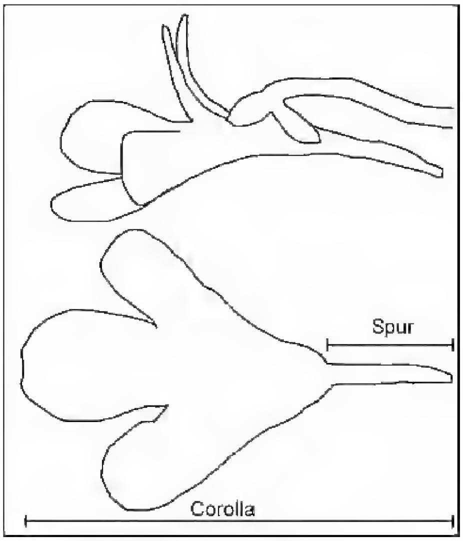

Figure 2: Outlines of typical flowers; the top outline is viewed from the side, the bottom is viewed from below. The bottom view is labeled to indicate the distances measured for the corolla length and spur length.

Method

The region around Gasquet, Del Norte County (California, USA) is arguably the population center for *Pinguicula macroceras* subsp. *nortensis*. Five sites in the area were studied during 3-4 May 2008. These sites are within 15 km of each other, but span a range of microclimatic conditions and soil characteristics.

Site 1: A gentle south-facing slope seepage in a sparse conifer woodland with loamy soil; 150 m a.s.l. Forty flowers sampled.

Site 2: A north-facing population of plants on the steep rocky banks of a small creek, soil occurs in small patches but reasonably well developed; 120 m a.s.l. Thirty flowers sampled.

Site 3: An east-facing roadside population of plants on a scree slope, the soil consists entirely of serpentinic chips; 145 m a.s.l. Thirty flowers sampled.

Site 4: A north-facing roadside population of plants growing on a sheer serpentine cliff; 135 m a.s.l. Two sub-populations were sampled at this location. The first sub-population ("4a") consisted of plants adhering directly to the barren cliff wall. The second sub-population ("4b") consisted of plants that grew on the pure serpentinic gravel at the cliff base. The second sub-population was clearly derived from individuals from the first sub-population, which had detached from the cliff wall and had become established on the scree at the cliff base. Thirty flowers were sampled from each sub-population.

Site 5: A north-facing population on a steep slope, the soil was pure serpentinic gravel; 45 m a.s.l. Two intermingling sub-populations were sampled. The first sub-population ("5a") consisted of normally pigmented plants. The second sub-population ("5b") consisted of plants with foliage that was suffused with brown pigmentation (*i.e.*, "chocolate plants."). Thirty flowers were sampled from each sub-population.

Castle Crags district: This north-facing population, investigated in RYM2008, occurs on wet serpentinic rock and scree with moderately well developed soil; 1840 m a.s.l. Thirty-four flowers were sampled by RYM2008.

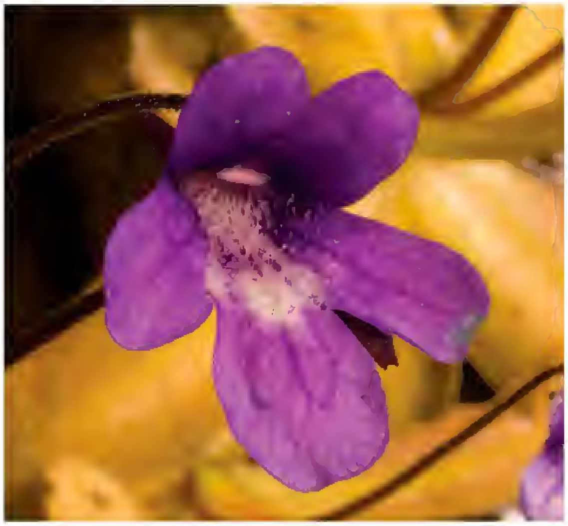

Figure 3: *Pinguicula macroceras* flower from Site 5b.

At each site, thirty or more flowers were selected (no more than one flower per plant). Measurements were taken of the length of their spurs (from tip to the point where the corolla tube flared; see Figures 2 and 3) and the entire length of the flowers (from spur tip, to distal margin of lower corolla lobe). Flowers were measured by gently lowering them onto the surface of a ruler, until the bottom of the flower was flat against the ruler; care was taken to avoid stretching the flower tissue. All measurements were made without damaging the plants or flowers.

Results

The averages and standard deviations for the spur and corolla lengths are given in Table 1. In his important paper on *Pinguicula macroceras*, Casper (1962) created histograms of his flower measurements (*i.e.*, numbers of flowers plotted as a function of corolla or spur length). In order to compare my data to his, histograms were created following his method. Specifically, Casper normalized each histogram so its peak reached a y-value of 1.0. He noted the x-points where the y-value of the histogram was equal to 1/2, and used these x-points to define the range of corolla lengths. Rondeau & Steiger (1997) do not explain how they developed their ranges in corolla or spur lengths; presumably they used the same method.

In RYM2008, character ellipses were used to study flower morphology. This method of presenting data, while novel and unconventional, neatly illustrates the two-dimensional data set (corolla lengths and spur lengths) for different sites simultaneously. To understand character ellipses, consider the data

Table 1: *Pinguicula macroceras* measurements

	Spur length (mm)		Corolla size (mm)	
Site/population	Ave± 1σ	From histograms	Ave± 1σ	From histograms
Site 1	8.1±1.0	(6)6.5-9.5(10)	26±2.7	(21)22.5-30(32)
Site 2	9.2±1.1	(7)8-10.5(11)	32±2.5	(27)29-35.5(38)
Site 3	5.7±0.8	(4)4.5-7	21±2.5	(15)19.5-24.5(26)
Site 4a (cliff wall)	7.0±0.9	6-9(9)	24±2.0	(22)22.5-25.5(30)
Site 4b (cliff base)	6.6±0.8	(5)5.5-8	27±2.6	22-31
Site 5a (typical plants)	6.8±0.9	(5)5.5-8(9)	26±2.4	(22)23-28.5(31)
Site 5b (brown plants)	6.3±1.0	5-7.5(9)	23±2.3	(18)21.5-24.5(28)
All plants (Sites 1-5b)	7.1±1.4	(4)5.5-8.5(11)	26±3.9	(15)22.5-28(38)
Castle Crags	6.6±0.7[1]	(1.5)6-8(9)[1]	25±1.4[1]	(17)24-28 mm[1]
Pinguicula macroceras	---	(1)6-9(11)[2]	---	(12)18-27(30)[2]

[1]Published/unpublished data from the study reported upon in RYM2008.
[2]From Casper (1962).

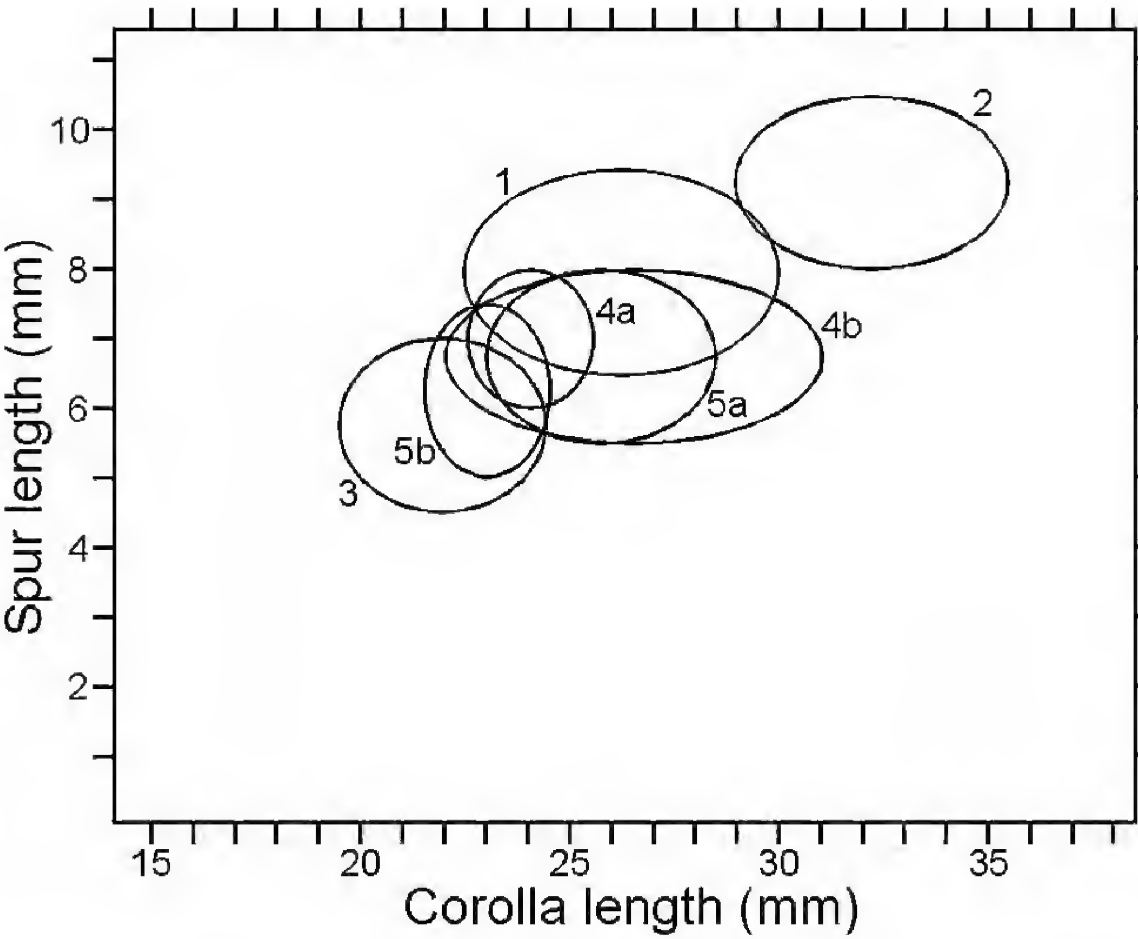

Figure 4: Inner range character ellipses for the seven populations of *Pinguicula macroceras* studied in Del Norte County. The different populations appear to exhibit distinct size ranges. In creating this figure, the vertical and horizontal major axes of each ellipse are set by the spur length range, and corolla length range, respectively. The values used are those for the inner ranges given for each character in Table 1 (*i.e.*, 6.5-9.5 mm for the spur length for the plants from Site 1). Each ellipse is labeled with population number, where each label is placed just outside its corresponding ellipse.

for Site 1 in Table 1. The histogram spur lengths and corolla sizes for Site 1 were 6.5-9.5 mm and 22.5-30 mm respectively. The character ellipse for this would be drawn so that the ellipse's vertical axis ranges from 6.5 to 9.5 mm, and the horizontal axis ranges from 22.5 to 30 mm (see Figure 4).

Discussion

The first question to be addressed is whether or not there are significant differences among the seven populations sampled in Del Norte County. Figure 4 shows the character ellipses for the spurs and corollas. Clearly, the spur and corolla lengths for each population fall in different places on this diagram. If these data were interpreted with an eye for detecting small differences, the populations might appear significantly different. It might even be argued they should be recognized as separate taxa. For example, without the data from Sites 1, 4, and 5 included, the plants from Site 2 and Site 3 look like two different taxa on this diagram, but they are not. The lesson here is that an inadequate sample of a population can result in the illusion that separate taxa exist, only because the intermediate plants had been overlooked.

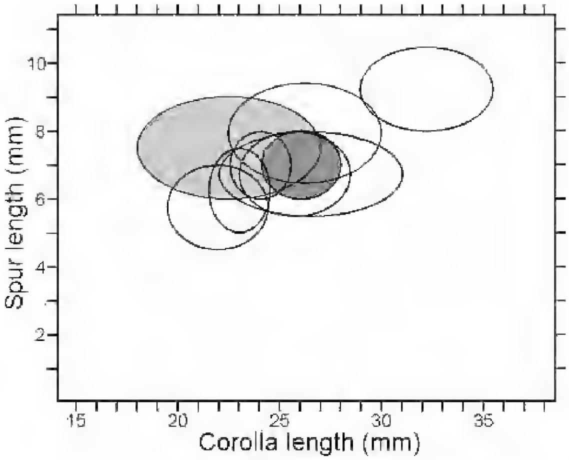

Figure 5: Data from Figure 4 repeated. Also shown are the inner character ellipses for the Castle Crags data (dark grey) and Casper's values for *Pinguicula macroceras s. lat.* (light grey). Notice that both are reasonably coincident with the range of *Pinguicula macroceras* in Del Norte County.

The second question asks are the site-to-site differences in flower characteristics purely genetic? The data suggest that differences are at least partly environmental—the populations with the largest flowers (*i.e.*, at Sites 1 and 2) occurred on well developed soils (*i.e.*, loam soils with large fraction of organic material), while the soils at the Sites 3, 4, and 5 consisted of pure, serpentinic gravel chips. The two sub-populations at Site 4 grew in different exposures (*i.e.*, on a cliff wall *vs.* on the flats at the base of the cliff), but the substrate for both sub-populations was the same pure serpentinic gravel chips; as such, there were no significant differences between their flower characteristics. This suggests that soil type may be more important than soil slope or wind exposure.

In order to look for genetic influences, reciprocal transplants or common greenhouse studies would be necessary, but that was beyond the scope of this study. Interestingly, while the two sub-populations at Site 5 were separated on the basis of leaf coloration (normal green leaves *vs.* brown leaves), there were no significant differences between their floral characteristics. However, there is a slight difference (the chocolate-colored plants tended to have slightly smaller flowers), and this could be an avenue of further study.

The third question asks how do the plants from the Castle Crags district fit into this analysis? Figure 5 repeats the data shown in Figure 4, but also includes character ellipses for the population from Castle Crags district (shaded dark grey; from RYM2008), and *Pinguicula macroceras s. lat.* (shaded light grey; from Casper 1962). It is clear that the Castle Crags plants fall well within the range of variability for plants in California. The flowers are not morphologically different from other Californian plants.

The fourth question asks if the data support the separation of *Pinguicula macroceras s. lat.* into two subspecies. The strongly overlapping character ellipses in Figure 5 clearly illustrate that there is no floral evidence to separate the recognition of the taxon *Pinguicula macroceras* subsp. *nortensis*.

While flower size was an important characteristic used in the original description of *Pinguicula macroceras* subsp. *nortensis*, it was not the only one. Another character was the shape and degree of overlap of the lower corolla lobes—large in *P. macroceras* subsp. *macroceras*, small in *P. macroceras* subsp. *nortensis* (Rondeau & Steiger 1997; Steiger 1975). In practice, the supposed degree of petal overlap is not readily visible (see discussion in RYM2008). Further, photographs of *P. macroceras* subsp. *macroceras* from Japan, both in the wild and in cultivation, clearly illustrate the lack of corolla overlap (Partrat 2005; Takai 1995).

Since floral size and corolla overlapping do not appear to be significant characteristics, the only remaining difference between the two subspecies is the nature of the tips of the calyx lobes. This attribute was addressed in neither the current study nor in RYM2008. However, such a minor detail surely does not merit subspecific designation. Therefore, I conclude that *Pinguicula macroceras* subsp. *nortensis* should no longer be recognized as a significant entity, and should be reduced to synonymy of *Pinguicula macroceras*.

The general philosophy of this discussion can be extended to other butterwort species of Europe and Mexico, which are sometimes the subject of contentious disagreement between lumping and

splitting botanists. The inherent variability of *Pinguicula* in Del Norte County is a reminder to proceed cautiously.

Acknowledgements: I wish to thank Elizabeth Salvia and Bob Ziemer for help in the field, Harry Tryon for guidance to sites I was unfamiliar with, and Jim Miller for his companionship in the field. I also thank Fernando Rivadavia for many useful suggestions that improved drafts of this paper. No permits were required to visit the public and unposted private locations described in this paper. No specimens were collected during the course of study.

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Pinguicula in the shadow of Mount Shasta, California

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Keywords: ecology: *Pinguicula macroceras*, Castle Crags Wilderness, California

I first learned of the presence of butterworts in the Castle Crags Wilderness area in the summer of 1995. An unexpected telephone call revealed the presence of butterworts in a rather obscure and heretofore unreported location over 100 km east or southeast of all previously reported sites in Siskiyou County, California or Josephine County, Oregon. Was this some kind of a hoax that some of my carnivorous plant buddies had manufactured?

Perhaps, but the possibility was tantalizing—butterworts so far from any known distribution. These plants were seemingly locked into a cirque and surrounded by colorful locales known as the Devils Pocket, Panther Rock, Bear Ridge, and Fawn Creek. Surely this would be worthy of a day trip even if it was only a simple diversion.

Initial research about the area revealed the majestic Mt. Shasta (4329 m /14,200 ft) to the east and the less imposing Mt. Eddy (2752 m / 9025 ft) to the north. Forest Service data indicated the area was a byproduct of Pleistocene glaciations with resultant serpentine soils in abundance (Keeler-Wolfe 1990).

Rare plants noted in the Forest Service Research Natural Area surrounding this site included a new species of manzanita (*Arctostaphylos klamathensis*), the cobra lily (*Darlingtonia californica*), a true lily (*Lilium washingtonianum* var. *purpurascens*), and a sedge (*Carex gigas*) (Keeler-Wolf 1982, 1989). Significant fauna included spotted-owl (*Strix occidentalis*), pine marten (*Martes americana*), wolverine (*Gulo gulo*), and mountain lion (*Felis concolor*). Yes, this certainly sounded as though it could be an interesting place to visit.

Alas, to get anywhere near this site, I would have to wait until July for the snows of winter to melt away. My approach to the site was on a paved road that turned into rutted gravel, which devolved into the glacial moraines alluded to above. Much later the various lakes came into view surrounded by intermittently forested cliffs.

The original site for *P. vulgaris* subsp. *macroceras* Link at 1829 m (6000 ft) near the Curious Lake[1] [PUA # xxxxx, 7/12/86] was noted by Glenn Clifton in streamside habitat. *P. vulgaris* subsp. *macroceras* is the official taxon for butterworts found in northern California and southern Oregon, although as many of you know *P. macroceras* subsp. *nortensis* was proposed several years ago by Rondeau and Steiger (1997). This name has now become widely dispersed in botanical journals.

Again in 1996, both Clifton and D. W. Taylor returned to this area, but noted the elevation as 1921 m (6300 ft) [JEPS #xxxxxx, 7/26/96] with both *Drosera rotundifolia* and *Darlingtonia californica* on serpentinized gabbro in "...a wet seepage area..." above Curious Lake. Later in this decade I would also determine the most obvious source of this "seepage", and its distinct relevance to my quest.

Field Research

In July of 1996 and seemingly well prepared with all this information, I faced the Curious Lakes complex for the first time and my first thought was: Where to now? There was no one "site" to sur-

[1]Note: Curious Lake(s) is a fictitious name that is used in this paper to protect the true identity of this private property site within the Shasta-Trinity National Forest. All collection numbers for herbarium specimens have been deleted to serve this same purpose.

vey, but rather only a series of circular vistas of rock and trees climbing skyward. Little did I know then that I would spend the next 10 years wandering in this wilderness.

My first two visits with carnivorous plant enthusiasts and off-road specialists Bruce Bonar and Stephanie Changaris turned into scoping and reconnaissance missions by both foot and boat primarily looking for access to seepy sites draining into Curious Lake. During this time various lakes were visited and searched for possible unreported sites or points of access that had not yet been found. Swimming across the lake was very refreshing, but ultimately unproductive for every potential take-off point found to higher seepy ground was now unreachable without boots. The surrounding area supported *D. californica* and *D. rotundifolia* abundantly and two species of *Utricularia*, but none of the butterworts that had previously been reported.

A few years later I enlisted the help of Arthur Yin and Gina Morimoto in the hope of bringing a fresh perspective to the mysteries of this site. Our initial visits were again unproductive, but in 2002 a key piece of information trickled down from the Forest Service: a compass heading at which these plants were said to have been seen. Finally, we had the missing link—a direction from which to launch out of the water onto the cirque.

In the late summer of 2005, Arthur and Gina did find one site for butterworts in the area previously predicted by Forest Service information and some of the other data we had compiled over the years. Unfortunately, that was in September, hence no flowers were available for a definitive identification. My review of specimens at Jepson Herbarium in 2006 indicated that the species found there appears to be a "macroceras" type, but of course I would love to see some fresh material for further evaluation. Barry Rice and Beth Salvia also visited this site in the fall of 2006, but of course it was too late again for any blooming material.

So it was in June 2007, as I had become Captain Ahab resolved to take just one more shot at what could have easily become my Moby Dick after enduring hot days, cold nights, thunder-lightning-hail on my head, and enough illusions to write a damn book. Using Arthur's stealth trail, I went around the southwest corner of the lake and slowly up the hill into the seepy area and had my Eureka moment—butterworts in eastern Siskiyou County!

Curiously, although I was the last one of our small group to reach this site, perhaps it was only fitting that I was the first to see these butterworts in bloom with associated species such as *Chamaecyparis lawsoniana*, *Arctostaphylous* spp., *Cornus stolonifera*, *Rhododendron occidentale*, *Leucothoe davisiae*, *Carex* spp., *Dodecatheon* spp., *Lillium* spp., *Phyllodoce empetriformis*, *Cassiope mertensiana*, *Platanthera sparsiflora*, and *Listera convallarioides*. *D. rotundifolia* was also reported to have been seen here in the past, and although it is quite abundant at lower altitudes, it has not been seen with these *Pinguicula* yet.

Observations & Conclusions

Now that I have been there and done that, does anyone care just what species might be lurking here? Or just why is it here at what Dean Taylor called a "...very crazy site..." apparently under our botanical radar for who knows how long? Of course, I believe that someone cares, so I have prepared some information for you about the two issues that must be considered regarding this site: taxonomy and plant distributions in general.

First, having seen these plants in bloom, and after completing a review of the limited statistical data available on calyx shape, corolla and spur length, it appears that this species is some form of *P. macroceras* Link as outlined in Table 1 (Casper 1962). Curiously, it just happens to occur on serpentine soil, where I have often seen it throughout its range in western North America. It is also

Table 1: Comparison *P. macroceras* and *P. vulgaris* W. North America (H. Rondeau 2007, ver. 1.1).

Element/ Characteristic	***Pinguicula macroceras***	***Pinguicula vulgaris***	**Comments**
Habitat:	Often petrophillous; esp. on serp. CA, OR (excl. Wallowa Mts.), WA (excl. Olympic Mts.)	Var. but usually lakeshore/floodplain	
Corolla length:	18-27	14-22	Casper (1962, 1966)
Corolla lobe shape:	Subobovate-oblong, edges touch/overlapping	Oblong, not covering nor touching	Casper (1962, 1966)
Spur length:	6-11	3-6	Split spur/s tip noted at CL (Rice *et al.* 2008), previous reports of same not known
Calyx:	Split up to ½ length	Split up to ⅔ length	Casper (1962, 1966)
Capsule:	Ovoid (Casper 1966)	Ovoid (Casper 1966)	*P. m. nortensis*: Globular (Rondeau & Steiger 1997)
Chloroplast-DNA	Appears to support Casper (1966), but see conclusions	Appears to support Casper (1966), but see conclusions	Note Cieslak *et al.* (2005)
Nuclear ribosomal ITS	Appears to support Casper (1966), but see conclusions	Appears to support Casper (1966), but see conclusions	Degtjareva *et al.*, (2006), Degtjareva (pers. comm. 2007)
Seed Shape:	Micropylar appendage absent	Micropylar appendage present	Degtjareva *et al.*, (2004), Degtjareva (pers. comm. 2007)
Trichomes (pubescence) in corolla tube. Three specific areas noted in Godfrey & Stripling (1961)	Casper (1966) Is variation significant?	Casper (1966) Is variation significant?	(Rondeau & Steiger 1997); *P. m. nortensis* only; significance noted in description

found at a record high altitude (>1890 m /6200 ft) for this species in both California and western North America.

Kruckeberg (1984) listed *Pinguicula vulgaris* L. in California as a bodenvag or indifferent taxon, while Alexander (2006) implicated *P. macroceras* as part of the " …California Pitcherplant Alliance…" (*Pinguicula*, *Drosera rotundifolia*, and *Darlingtonia californica*). Certainly these species are often found on serpentine throughout northwestern California and southwestern Oregon, but

this group is rarely seen together in the field. Rondeau and Steiger (1997) had noted high serpentine affinities for our *P. m.* subsp. *nortensis* taxon and other researchers (Walker 1954; Whittaker *et al.* 1954; Kruckeberg 1984; Brooks 1987) have had much to say about this edaphic phenomenon as well.

However, the current the state of research on *Pinguicula* (Degtjareva 2002, 2007; Degtjareva *et al.* 2004, 2006; Cieslak *et al.* 2005) indicates that perhaps a synthesis of our western species is at hand. My reading of the current research, discussion with one of the primary authors involved, and in consideration of current trends in angiosperm phylogeny (Stevens 2007) used in the Flora of North America, appears to show that *P. macroceras* and *P. vulgaris* are merely sisters under their skins. I think that the species name *macroceras* should now be applied to all *Pinguicula* from California to British Columbia and Montana with possible extensions of this name to both Alaska and Japan in the future.

One expert (Rivadavia 2008) has offered a note of caution on this conclusion, stating that the most definitive test on the DNA of all species concerned has not yet been performed. Certainly, additional research should and will be done in the future regarding both DNA and seed characteristics as has been suggested by Rivadavia (2008) and outlined by Cieslak *et al.* (2005), Degtjareva (2002), Degtjareva *et al.* (2004, 2006), and Soltis *et al.* (1997) to further clarify these taxonomic issues. Looming on the horizon, there is even the possibility that DNA barcoding of plants may be a possible source of determining taxonomy (Cameron 2007; Hollingsworth 2008).

Second, is this isolated population simply a quirk of fate as some seeds fell out of the sky via avian transport—avichory, as migratory golden plovers have been implicated in the creation of the disjunct population of *Drosera anglica* to the Alakai Swamp in Hawaii (Gon 1994)? I have seen both spotted sandpipers and lesser yellow-legs in many western fens picking their way through both plants and fruits. Perhaps they were transported here from some closer, yet unknown site by mammals—zoochory (Molau 1990), or through simple aquatic transport—hydrochory (Danvind & Nilsson 1997).

Also, as has been suggested by more than one botanist, is this apparently disjunct site actually relictual—was *Pinguicula* once far more widespread in California or Oregon than it is now? Have these plants been evolving here long enough for speciation to have already occurred, and where will the next disjunct population of *Pinguicula* be found? For those who may wish to jump into this exciting field work, I would encourage you to concentrate on the following areas: Gray Rock Lakes, Mumbo Basin, Gumboot Lakes, Picayune Lake, Marble Mountain, Trinity Alps, and the Siskiyou Wilderness.

Of course, the question of global warming or global climate change must also be considered as other plant populations are already climbing uphill, blooming earlier, or simply going extinct in their attempts to escape some of these effects (Lesica & McCune 2004; Malcolm *et al.* 2006; Banks 2006; Nijhuis 2007; Primack & Miller-Rushing 2007). The current projection for much of northern California is increasing the amount of winter precipitation that falls as rain, not snow, as is normal here from late October through March. It will be interesting to see just how this will affect the butterworts at Curious Lake.

N.B. 1. Note of caution to all who might follow in my footsteps: This area is private property located within the national forest, and it is a potentially dangerous area—footings are irregular and unpredictable; use of handholds on both rocks and trees on slopes is highly recommended.

N.B. 2. Additional research on other special interest plants is also being conducted on this vast area south and west of Curious Lakes known as the Trinity Ultramafic Region (James *et al.* 2002).

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The Savage Garden: "The creation and early evolution of CPN"

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Keywords: biography: Don Schnell, Joe Mazrimas

"I have been wondering about developing a little mimeographed newsletter dealing with carnivorous plants to be compiled and mailed quarterly."

Thus wrote Don Schnell to Joe Mazrimas on January 23, 1970. He continued: "This could really develop into a clearing house of information, hints, sources, and summary of interesting work being done. One of the key features would be a bibliography with a brief summary and critique of each paper. Also, each subscriber could send in a few paragraphs of interest, although we could not print long papers. I have seen this done in other fields and the results seem to be gratifying. Let me know what you think."

Over a year and a half later, on August 27, 1971, Don and Joe brought up this idea once again. "The need for some sort of organization does keep cropping up in my head, too," Don wrote. "It would be an awful lot of work, but it keeps bugging me." He mentions a ten-year-old newsletter called "Bat Research News" put out by the "bat people," a format he thought they could emulate. It was shortly thereafter that CPN was born, and at times Joe and Don nearly went batty!

* * *

Figure 1: Joe Mazrimas (left) and Don Schnell (right) in 1999.

On November 15, 1971, Joe sent a sample introductory newsletter to Don, called "Amphora," which was Greek for "jug or pitcher." A few days later, Don wrote back, suggesting a more obvious title "Carnivorous Plant News," which could also be abbreviated into CPN. Joe agreed. It should be extremely amusing to the current editors of CPN to read about Don and Joe's early headaches getting the first announcement of their publication off the ground. This was a time before common Xerox machines, computers, and email.

On January 23, 1972, Don wrote, "It seems we better get the CPN going. I contacted a local office supply store that does mimeograph work on the side. They can give us a hundred copies of our initial one-page newsletter for $1.84 … I will bear the printing cost, I think that is about as reasonable as we will find until we get fancy later on perhaps and go to offset if the thing gets big… My calculations indicate that a four sheet newsletter printed on both sides would cost 12

cents apiece to print. Mailing is 8 cents more, thus 20 cents per CPN on the way to the subscriber." They decided to charge an annual subscription rate of $1.00 in North America, and $2.00 for foreign subscriptions. The extra pennies would pay for things like staples.

* * *

The first time Joe, who lived in northern California, and Don, who lived then in Atlanta but later moved to North Carolina, contacted each other was in June, 1968. Don heard that Joe collected carnivorous plants from Warren Stoutamire, a botanist at the University of Akron in Ohio, and wrote Joe a letter introducing himself. Thus began a long history of letter writing, trading plants, and gossip. By 1972, they had a small circle of carnivorous plant enthusiasts, including notables at the time like Ritchie Bell at the University of North Carolina, Chapel Hill.

To get out word of the newly germinating CPN, they printed 300 copies and sent them to the few enthusiasts known personally to them, plus copies to the authors of CP research articles found in botanical publications, university botany departments, botanical gardens, and a few hobbyists they had been in contact with. Don sent 200 of the announcement issues through the eastern half of North America plus Europe and South Africa. Joe sent 100 throughout western America, plus Australia and Asia. Don wrote to Joe on February 8, 1972, "I might even have included Howard Hughes if I was certain he still existed." They planned the first issue for that April.

There were numerous annoying little problems they had to resolve before subscriptions came in. Don's secretary enthusiastically did all the typewriting. ("She doesn't have THAT much to do in the office, but don't tell her that!") Addresses of subscribers were kept on index cards and each new subscription had four address labels made so they would know when each annual subscription ended. At first, no photos, but some line drawings were used.

On February 25, 1972, Don wrote, "So far, I have received only one reply to our mass mailing, besides Ritchie Bell and Warren, three all together." He was hoping for 10 to 15 more.

Ritchie Bell offered use of the university's printing department, which saved time and several pennies per issue. By March 7, Don wrote, "I think CPN is catching on, I received two more subscriptions Monday, one from Kew Gardens and one from Canada." By March 11, they had 16 subscriptions. "In a very few years we'll have hundreds."

Ironically, Katsuhiko Kondo was doing research at UNC-Chapel Hill at this time, working with Ritchie Bell. Katsu was the son of Masahiro Kondo, and both were CP enthusiasts. Katsu's book "Carnivorous Plants" was scheduled to be published in Japan in 1974, and ten years later, father and son's "Carnivorous Plants in Color" would be very popular, especially for its many photos of rare CP at the time. It was Katsu who designed the lovely original masthead illustration of a cobra plant, hooded pitcher plant, Venus flytrap and thread-leaved sundew with the bold title "Carnivorous Plant Newsletter."

Joe and Don were astounded when they found out Japan had an "Insectivorous Plant Society" since 1949, and at the time had 300 members! They had been publishing their own newsletter for twenty-three years!

Katsu announced the new CPN in the Japanese newsletter, and Don and Joe were quite befuddled when subscriptions poured in, but all the correspondence and addresses were in un-readable (to them) Japanese. They resolved this by sending a bulk mailing to Japan's society, who then distributed them to their members.

* * *

The first issue of the Carnivorous Plant Newsletter was published on schedule in April, 1972. It was 15 pages long. There were about 25 subscribers. After an introductory "Editors' Corner" welcoming everyone and mentioning future plans, the "New Subscribers" section gave their names, addresses, and a few sentences about their interests in CP. Most were teachers and researchers from university botany departments, but also members from public gardens and businesses subscribed, such as Kew Gardens in England, Longwood Gardens in Pennsylvania, and the Carolina Biological Supply Company. There were very few "hobbyists" at this time. Four informative articles appeared in the first issue. Warren Stoutamire wrote about monitoring salts in soil and water, Joe wrote about *Drosophyllum* and its difficulties, plus a piece on *Chrysamphora* (*Darlingtonia*), and Don wrote about *Sarracenia oreophila*. The rest of the issue was a "catch-up" on recently published scientific literature and a brief review of Rica Erickson's 1968 book "Plants of Prey," about Australian CP.

By June 29, just as the second issue in July was published, CPN had over 100 subscribers, nearly half from Japan. The excitement also had its stress. Good doctor that he is, Don told Joe to "take your vitamins and get plenty of rest." The second issue added the ever popular "News and Views" section, plus a list of a dozen carnivorous plant suppliers, all but one in the United States.

* * *

For anyone who has never sat down with either hard copies of CPN's early years or read them online in the Society's archives on its website, you're missing out on hours of engrossing, informative history that can become quite addictive. So many subjects were covered so rapidly as the subscriber base expanded from the U.S., Europe, and Japan to include growers in Australia and South Africa, early CP enthusiasts at the time had their brains nearly explode (not to mention their greenhouses, terrariums, and bog gardens). The modern phase of knowledge about these incredible plants had begun, and we owe it all to Don Schnell and Joe Mazrimas.

* * *

Joe Mazrimas was born in Boston in 1937, and graduated from Boston College 21 years later. He moved to California "in search of gold!" and graduated in 1963 with an M.S. in Biochemistry from the University of California in Davis. It was around this time two things happened that would change his life. He killed a couple of Venus flytraps bought in a local nursery with Davis' notoriously hard tap water, and met and married his wife Kathy. In the library, Joe found out some basic information on how to keep flytraps alive. He worked at UC Davis a few years, then transferred to the Lawrence Livermore Laboratory east of San Francisco. He worked "with DNA, proteins, lipids as well as synthesized peptides" for 35 years. He retired in 1997. He and Kathy have two kids and four grandchildren, all of whom have black thumbs!

I first met Joe in 1984 when I displayed plants in my collection at the San Francisco Plant and Flower Show. There were a handful of CP growers in the Bay Area at the time, which we later organized into the Bay Area Carnivorous Plant Society. I had been growing CP since junior high school but had only discovered CPN a few years before meeting Joe. It was a humbling experience hanging out and trading plants with one of the creators of CPN. I went on several fun field trips with Joe and his best buddy Larry Logateta. I have always found Joe to be child-like in his joy and enthusiasm growing carnivorous plants, completely unpretentious, and always willing to share his encyclopedic knowledge, especially about the early days of the modern hobby. Shortly after his retirement he and Kathy drove up to Sonoma County where I live in a resort town to show off their huge RV, which

they have used to explore CP sites from California to Maine. His stories of those trips are delightful. Naturally, Joe is still active in BACPS and maintains a huge collection. He's won many awards for his plants.

* * *

Don Schnell was born in 1936 and raised in Toledo, Ohio. His dad worked for the railroad, and seeing young Don's interest in chemistry, zoology, and botany, steered him into medicine. In college, Don began to explore local bogs looking for CP, and he graduated with a medical degree from Ohio State and moved on into pathology. He spent several years as a pathologist in the Army, and was happy to be finally stationed at Ft. McPherson in Georgia, where he spent most weekends looking for CP in the southern savannahs.

(Ironically, a middle-aged guy came into my nursery, California Carnivores, in the 1990's and enthusiastically recalled a doctor when he was in the Army who would take soldiers on field trips to see CP. I held up a copy of "Carnivorous Plants of the United States and Canada" and exclaimed, "You mean Doctor Don!?" The guy freaked out!)

After the Army, Don got a job as a pathologist in a small civilian hospital in North Carolina, where he met his wife Brenda, a lab technician. They have two daughters, also in the medical field, and a few grandkids who get a kick out of Don's CP collection. Don's "other hobby" is outdoor model railroads. He loves his trains. He finally retired only three years ago ("I should have done it ten years ago!")

I have a few memorable experiences with "Dr. Don," as I call him. He first contacted me in the late 1980's looking for a few plants like *Drosera regia*. In return, he sent me **boxes** of *Sarracenia*, many type-specimens, from his collection. All of these plants are part of California Carnivores' stock and display collection. He also sent me all of his published botanical papers.

A funny memory was my first time meeting him at a presentation I did at the University of North Carolina in Chapel Hill. I had no idea what Dr. Don even looked like, but Marie Baumgartl, who owned "Marie's Orchids and Carnivorous Plants" in Florida back in the 80's and 90's, warned me, "He looks like a handsome military drill sergeant!"

When he first shook my hand, I think I winced and my knees buckled. This was the man who taught me the word "salubrious." I also remember Don's laughter in the audience at my CP jokes.

Needless to say, Don is the author of the two editions of "Carnivorous Plants of the United States and Canada" (1976, 2002). But he's not done yet. He is working with Stewart McPherson on a two volume monograph of Sarraceniaceae, due to be released soon.

After years of editing CPN, it is ironic Joe and Don never met in person until the first ICPS convention in Atlanta in 1997. I witnessed it. It was a joyous event.

* * *

Thanks to Don and Joe for helping me with this article, with particular appreciation to Joe, who gave me a stack Don's old typewritten letters! Thanks also to Liz Brown for technical assistance (typing).

PDFs of all issues of CPN from 1972 to present are available to ICPS members at http://icps.clubexpress.com. Individual articles printed in CPN are publicly available at http://www.carnivorousplants.org/cpn/Search.php.

Carnivorous plant protection project in Korea

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Keywords: conservation: biodiversity, ethics, Korea

As all carnivorous plant lovers are aware, carnivorous plants inhabit wetlands. However, due to urban encroachment and people's neglect to the environment, wetlands are at risk. This article is written to raise awareness of how we should take initiative in protecting carnivorous plants and the wetlands they live in.

As a zealous carnivorous plant lover, I work in the Korean Carnivorous Plant Institute where mass-propagation is used to prevent extinction of carnivorous plants. Mass-propagation is the technique of taking parts of plants to mass-produce them. For instance, plants like *Utricularia bifida* L. and *Utricularia racemosa* Wall. are carnivorous plants that used to be endangered in Korea. Because mass-propagation (following the thesis written by Dr. Gi-Won Jang) was practiced, they were able to be rescued.

In order to stop the extinction of other carnivorous plants in Korea, I traveled to different wetlands to actively find and protect them. One of my meaningful journeys was to Yongneup (see Figure 1).

Figure 1: Yongneup, Mt. Daeam, South Korea.

Figure 2: Max and his father in Yongneup, taking a break after finding the carnivorous plants.

Figure 3: *Drosera rotundifolia* at Yongneup, Mt. Daeam, South Korea.

Figure 4: A red form of *Drosera rotundifolia* at Yongneup, Mt. Daeam, South Korea.

Yongneup, literally translated as "Dragon Wetland," is located in Mt. Daeam, South Korea. It is one of the wetlands included in the Ramsar List of Wetlands of International Importance (Ramsar Convention is the international treaty dedicated to protection of wetlands). Public access is strictly controlled. In fact, Yongneup is located in the DMZ (de-militarized zone) between North and South Korea. Thus, I had to obtain a permit from the government to enter the wetland to collect and mass-propagate the carnivorous plants that are unique in South Korea.

In Yongneup, I was able to find typical plants of *Drosera rotundifolia*, and also a type of *Drosera rotundifolia* which has pink flowers and more reddish colored leaves than is typical. This species is endangered in Korea (see Figures 2-4). Even though *Drosera rotundifolia* was once prevalent in wetlands in Korea, years and years of development of wetlands eventually made it an endangered species. They are even on the verge of extinction at the protected sites in Mt. Daeam. Therefore, in order to protect and preserve the biodiversity, and with my permits from the government, in July 2010, I collected seeds from a site at approximately 38.22ºN, 128.10ºE, 1100 m a.s.l., and brought them back to the Carnivorous Plant Institute (see Figure 5). These details of collection location are provided with the knowledge that the site—in the DMZ—is certainly not at risk of poaching impacts.

Figure 5: Max and Dr. Gi-Won Jang in the Carnivorous Plant Institute, preparing for mass-propagation.

Figure 6: Max preparing for implantation of *Drosera rotundifolia*.

Then, I mass-propagated and replanted the plants in other wetlands in Korea, near Ogok-dong and Pado-ri (see Figure 6). Dr. Gi-Won Jang and I had to carefully browse through the wetlands where these two carnivorous plants had already existed in the past and been destroyed because of urban encroachment. We selected two sites for re-introduction of live red and normal *Drosera rotundifolia* plants: Site 1 is a wetland in Bogil Island, Bogil-myeon, Wando-gun, Jelloanam-do, South Korea (34.16ºN, 126.55ºE; 15 m a.s.l.) and Site 2 is a wetland in Mt. Wolchul, Gaesin-ri, Yeongam-eup, Yeongam-gun, Jeollanam-do, South Korea 34.77ºN, 126.71ºE; 390 m a.s.l.). Such introductions must be done with great concern and responsibility. Despite the merits of being able to see carnivorous plants in nature, it is generally advised to not introduce exotic species. Planting carnivorous plants from different geographical locations can lead to genetic drift and introduction of pests and invasive species which can drastically alter the ecosystem.

While this location information exposes the plants to possible poaching risk, it is more important that future botanists encountering the plants will know that they were introduced. Furthermore, if the plants happen to spread naturally to other sites, providing explant details now will allow future scientists to analyze the spread properly.

I wish more people were aware of the importance of carnivorous plants, protect the biodiversity of wetlands, and take actions with high levels of responsibility.

A vacationer's guide to carnivorous plant locations

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Keywords: travel: *Drosera, Sarracenia purpurea, Darlingtonia californica*

In 1961, National Geographic Magazine published an article by Paul Zahl (1961) entitled "Plants that eat insects." This was followed by a related article (Zahl 1964) entitled "Malaysia's giant flowers and insect-trapping plants." Although I was just a young teenager at the time, these two articles introduced me to the fascinating and bizarre world of carnivorous plants, and created a curiosity in me that has persisted to this day. In the first article, Zahl discussed the world of Venus fly traps, sundews and American pitcher plants and created a bog terrarium of carnivorous plants that came from the only company at that time from which one could easily obtain these, *i.e.*, the Carolina Biological Supply Company. In the second article he wrote about the more exotic pitcher plants from Borneo in the *Nepenthes* family.

It wasn't long after that that I placed an order and created my own bog terrarium with these fascinating plants. Although these lived only a short period a time, it set the stage for further purchases over many years that peaked with the successful growth of *Nepenthes* plants in my basement under halogen lights. Although I have often thought of field trips to see carnivorous plants growing in their natural location, the demands of career and family have kept me from doing this. In addition, it has appeared to be very difficult to find the exact locations of such plants because of their endangered status. I have subscribed to Carnivorous Plant Newsletter for nearly two decades and have always

Figure 1: View of Bog Trail from boardwalk in Pacific Rim National Park, Vancouver Island, Canada.

Figure 2: *Drosera rotundifolia* among sphagnum moss as seen along Bog Trail.

noted how circumspect the authors are in describing the location of their finds, although I certainly understand and agree with their concerns.

However, during the last three years, three distinct opportunities unexpectedly arose in which I was able to observe and photograph different species of carnivorous plants in their natural settings, without the need for any special permits, hiking through bogs or swamps or hours spent researching possible locations. By sheer coincidence, during vacations taken for other reasons, I found carnivorous plants growing in the wild! Not only are the sites themselves not secret, but in fact are well publicized in literature available at the parks. These locations are also easily accessible on well-maintained trails and present good photo opportunities.

The first site is in the Pacific Rim National Park on Vancouver Island in Canada. This is a magnificent temperate rainforest situated on the west side of Vancouver Island on Highway 4 between Ucluelet and Tofino. Located along the main road connecting these two cities and inside the national park is a trail clearly labeled, "Bog Trail." This trail consists of an easy hike on a boardwalk over a swamp that is in fact handicapped accessible. Scattered through this bog, with many growing right along the side of the trail, are thousands of *Drosera* plants. My wife and I visited this park in August, 2007, and it wasn't until we got there and I read the park's brochure on the trails that I knew about the presence of these plants. Figure 1 gives an overview of the bog as taken from the boardwalk and Figure 2 is a close-up the sundews growing along the side of the trail.

The second site also was not known about in advance, and was discovered when we visited Algonquin Provincial Park in Ontario, Canada in May, 2008. This park is located in northern Ontario along Highway 60 and is a nature lover's delight with an abundance of wildlife and trails. Near the east end of the park is a trail, clearly labeled "Spruce Bog Boardwalk." Like the previously mentioned trail, this is an easily hiked boardwalk that covers a large bog/swamp. The park's brochure gives the location where *Sarracenia purpurea* plants can be seen and photographed from the trail

Figure 3: Overview of bog as seen from Spruce Bog Boardwalk in Algonquin Provinicial Park, Ontario, Canada.

Figure 4: Close-up of *Sarracenia purpurea* growing near trail.

itself. Figure 3, gives a view of the bog as seen from the boardwalk, while Figure 4 is a close-up of plants growing near the edge of the trail. Note that due to the early time of the year there (May), the plants were only just beginning to come out of their winter dormancy.

Figure 5: Darlingtonia Wayside Park north of Florence, Oregon on US Highway 101.

Figure 6: Field of *Darlingtonia californica* growing near trail in park.

The third and final location was easily the most spectacular of all. This site is located approximately five miles north of Florence, Oregon on US Highway 101 and is in a small state park named Darlingtonia Wayside. This park consists of a small circular loop boardwalk, and at the midpoint of the boardwalk are hundreds, if not thousands, of mature specimens of *Darlingtonia californica*.

Many of these are nearly three feet in height, and we had the pleasure of visiting this site in August, 2008. The sign at the park entrance (see Figure 5) clearly states the presence of the many plants visible for viewing and picture taking (see Figure 6).

These three sites demonstrate that the casual vacationer with an interest can find many locations where carnivorous plants are available to view in accessible locations and without the need for any special permits. Also, as the sites are all advertized as having carnivorous plants by the parks themselves, the concern of maintenance of secrecy for the location is avoided. My final hope is that sometime while I am vacationing in the Southeast portion of the United States that I am able to find similar viewing locations for the Venus fly trap!

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Fluorescence tagging of phosphatase and chitinase activity on different structures of *Utricularia* traps

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Keywords: physiology: *Utricularia* — chemistry: digestive enzymes.

Introduction

The genus *Utricularia* (Lentibulariaceae) comprises about 220 species and represents the largest genus of carnivorous plants (Taylor 1989; Guisande *et al.* 2007). The trap of *Utricularia* is a hollow utricle usually 1-4 mm long, with walls mostly two cells thick, and filled with water. It is the most sophisticated trap within carnivorous plants (Juniper *et al.* 1989), with a variety of glands and trichomes both on the inner and outer surfaces (see Figure 1). After the prey irritates trigger hairs situated close to the trap door, it is sucked in as a result of the underpressure maintained inside the utricle. After firing, the trap restores the underpressure by rapidly removing approximately 40% of the water from the lumen. This process lasts about 30 min and the trap is ready to fire again (Sydenham & Findlay 1975).

Little is known about the mechanisms of digestion in *Utricularia* traps although protease, acid phosphatase, and esterase were detected cytochemically in the quadrifid glands (for review see Juniper *et al.* 1989). Recently, Sirová *et al.* (2003) investigated fluorometrically the activities of five extracellular enzymes directly in the trap fluid from four aquatic *Utricularia* species. Phosphatase always exhibited the highest activity, while the activities of the other enzymes were usually lower by one or two orders of magnitude and could have entered the trap from the ambient water. Furthermore, the trap enzyme activities are independent of prey digestion and the enzyme production is probably constitutive, *i.e.*, not induced by prey, unlike Droseraceae traps (*cf.* Juniper *et al.* 1989). Phosphatases represent a broad group of enzymes catalyzing the hydrolysis of phosphate esters. Acid phosphatases are common plant enzymes of low substrate specificity that appear to be important in the production, transport, and recycling of phosphate (Duff *et al.* 1994).

The importance of phosphatase activity in trap digestion also was supported by Płachno *et al.* (2006), who proved the presence of phosphatases using the ELF (enzyme-labeled fluorescence) method both inside the terminal gland cells (arms) and on the surface of quadrifid glands in traps of all 26 *Utricularia* species investigated. Although trap hydrolytic enzyme secretion is accepted as one of several principal criteria for plant's carnivorous syndrome (Juniper *et al.* 1989), the ELF method still remains problematic and partly unreliable as to the exact interpretation of data (*cf.* Sirová *et al.* 2003; Płachno *et al.* 2006). As each plant cell contains phosphatases in its cytoplasm or organelles, it may not be clear (*e.g.*, due to endocytosis of the ELF substrate) that the positive ELF reaction visualizes *strictly* the externally released or bound enzyme. Furthermore,

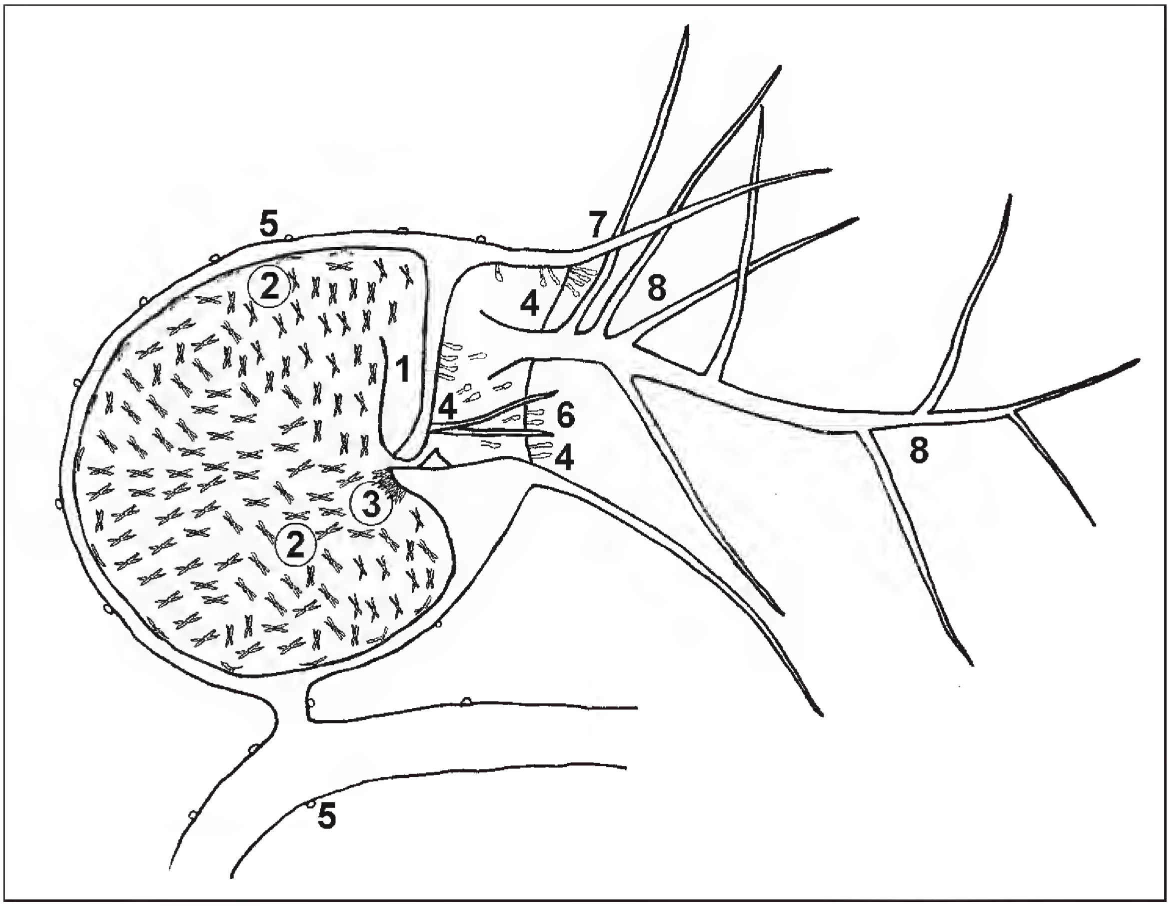

Figure 1: Schematic longitudinal section through a trap of *Utricularia* with glands and other structures (modified from Juniper *et al.* 1989). 1: trap door, 2: quadrifid glands, 3: bifid glands, 4: stalked mucilage glands, 5: spherical sessile glands, 6: trigger hairs, 7: rostrum, 8: antennae.

Płachno *et al.* (2006) used 12.5 times more concentrated substrate than the former authors did, which could be the reason for the difference in the extent of positive ELF results between the two studies.

Utricles are also inhabited by diverse communities of microorganisms (*e.g.*, Richards 2001). Considering that aquatic *Utricularia* species frequently grow in P-limiting conditions, that trap phosphatase activity is invariably high (Sirová *et al.* 2003), and that the affinity of phosphate uptake of some microorganisms (bacteria) is at least one order of magnitude greater than that of phytoplankton (Currie & Kalff 1984), it is highly probable that this microbial community enhances P acquisition for the trap under nutrient-poor growth conditions. Moreover, it has been found recently (Sirová *et al.* 2003, unpubl.; Płachno *et al.* 2006; Płachno & Wołowski 2008) that bacteria, cyanobacteria, and algae in the utricles and relative *Genlisea* traps also show extracellular phosphatase activity.

The aim of our study was to detect extracellular phosphatase and chitinase activity in traps of nine *Utricularia* species and distinguish it within the trap structures (*e.g.*, glands), commensal organisms, and periphytic organisms attached externally on the traps. Fluorescence tagging was used to visualize both enzymes. In four aquatic *Utricularia* species, the enzyme activity was investigated in intact traps after two days of prey capture. The greatest attention has been focused on aquatic *Utricularia* species as they have large traps.

Table 1: Summarization of phosphatase and chitinase activity found on different trap structures (for explanation see Figure 1), commensal (C, in trap fluid) or epiphytic (E) microorganisms. The numbers indicate structures, on which any (*weak*, medium, **high**) activity was detected; n.d.--not determined. Dotted line separates aquatic and terrestrial species; note that traps of the former were tagged as intact, but the latter as halved.

Species	Traps without prey		Traps with prey after 2 d	
	phosphatases	chitinases	phosphatases	chitinases
U. vulgaris	2, *3*, 5, *E*, *C*	E	2, **4**, 5, E	*8*, *E*
U. australis	2, 3, **4**, *C*	*8*, *E*	*3*, 4, 5, E	*8*, *E*
U. floridana	**2**, **4**, 7, *C*	**8**, *E*	**4**, **5**, 6, 8, E	8
U. intermedia	**4**, **5**, *C*	*8*, C, *E*	**4**, **5**, 8, E	*5*, 8, *E*
U. stygia	1, **4**	*8*	n.d.	n.d.
U. bremii	*2*, 6	*6*, *8*, *E*	n.d.	n.d.
U. sandersonii	1, *2*, **4**, **5**	**4**, **5**, 8	n.d.	n.d.
U. livida	2, 4, 8	4, 5, 8	n.d.	n.d.
U. reniformis	4, **5**	2, E	n.d.	n.d.

Materials and Methods

All plant species were obtained from the collection of the Institute of Botany at Třeboň, Czech Republic. Aquatic species, *Utricularia vulgaris* L. (from Czech Rep.), *U. australis* R.Br. (Czech Rep.), *U. intermedia* Hayne (Czech Rep.), *U. stygia* Thor (=*U. ochroleuca s.l.*; Czech Rep.), and *U. bremii* Heer *ex* Kölliker (Lake Oniega, NE Russia) were grown in plastic containers outdoors, *U. floridana* Nash. (N Florida, USA) in a greenhouse aquarium, and terrestrial species, *U. sandersonii* Oliver (South Africa), *U. livida* E.Mey. (South Africa), and *U. reniformis* A.St.-Hil. (Brazil) in a greenhouse terrestrial culture.

Both enzymes, phosphatases (phosphomonoesterases) and chitinases (β-N-acetylhexosa-minidases), were fluorescently tagged in intact traps without prey in six aquatic species (*U. vulgaris*, *U. australis*, *U. intermedia*, *U. floridana*, *U. stygia*, and *U. bremii*). Several leaves with intact traps cut from 11th and 12th adult leaf whorls (intermediate age, see Sirová *et al.* 2003) on shoots of *U. vulgaris* and *U. australis*, from younger parts of green photosynthetic shoots of *U. bremii*, or pale carnivorous shoots of *U. intermedia, U. stygia*, and *U. floridana* were used. The cuttings were thoroughly rinsed. Four excised intact traps without prey were transferred to the solution of 0.1 mM Trisma buffer (pH 7.5; *cf.* Štrojsová & Vrba 2006), supplemented with a substrate, and filled twice, successively after two firings after 1 h each by means of mechanical irritation. ELF 97 phosphate and ELF 97N-acetylglucosaminide were applied (at 20 μM final concentrations) for tagging phosphatase and chitinase sites, respectively. The filled traps were then thoroughly rinsed with distilled water and were transferred to the solution of 0.5 mM KCl + 0.02 mM $CaCl_2$. They were kept at 23-25°C in very weak daylight for 2-3 h until the microscopical evaluation (see below). The trap fluid from other 6-10 traps was collected by a glass pipette (for details, see Sirová *et al.* 2003), allowed to desiccate on a cover slip, and microscopically inspected for enzyme tagging of commensals, such as protists and bacteria.

Parallel leaf or shoot cuttings of these species (except for *U. stygia* and *U. bremii*) were placed into plastic jars (0.2 l) with culture water, fine zooplankton (*Chydorus* sp., Cladocera, size 0.5-0.6

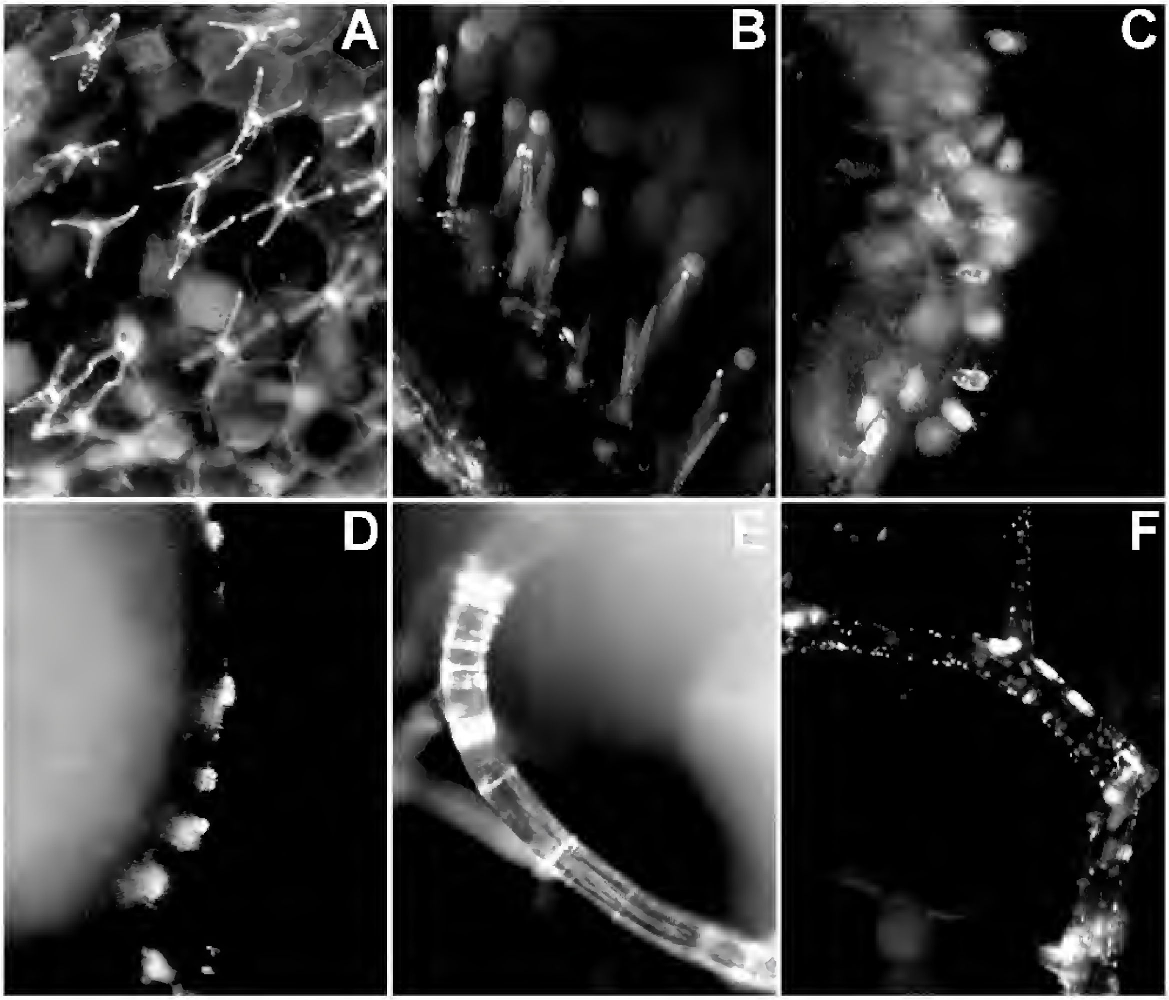

Figure 2: Examples of fluorescently tagged phosphatases (Ph) or chitinases (Ch) on different trap structures of *Utricularia* species. A: quadrifid glands (Ph, U. *floridana* without prey); B: bifid glands (Ph, *U. vulgaris* without prey); C: stalked mucilage glands (Ph, *U. intermedia* with prey); D: spherical sessile glands (Ph, *U. sandersonii*); E: antennae (Ph, *U. floridana* without prey); F: antennae (Ch, *U. floridana* with prey). Note distinct pattern of the enzyme tagging in E and F (dots likely tag enzymatic activity of epiphytic microorganisms, *e.g.*, suspect bacteria).

mm) was added, and carefully removed after 4-10 h. By this time, most of the traps contained prey. The cuttings were thoroughly rinsed and exposed in the filtered (mesh size 44 μm) culture water at 20-26°C (68-79°F) in dim daylight for 2 d. Afterwards, four excised intact traps with prey were filled twice by the solutions and further treated as described above for the empty traps. The traps of all aquatic species used were 1.5-3.5 mm large.

Before microscopic evaluation, the traps were halved lengthwise by a razor blade and thoroughly rinsed with distilled water. They were inspected with an epifluorescence microscope (Olympus BH-60, magnification 40-100×) and the images in green fluorescence were recorded with a monochromatic camera (for more details, see Štrojsová & Vrba 2006). Eight halves of four traps, either with or without prey, were inspected for each enzyme in all plant species. The attention was paid not only to trap structures but also to various attached trap organisms (periphyton).

Four traps without prey of three other terrestrial species (*U. sandersonii*, *U. livida*, and *U. reniformis*) were lengthwise halved by a razor blade, thoroughly rinsed with distilled water, put into the

same solution of either substrate as described above for 2-3 h, and were observed in the same way. The traps of the terrestrial species were only about 1 mm large.

Results and Discussion

Both phosphatase and chitinase activities were detected in all nine *Utricularia* species under study (Table 1). The brightest fluorescence, tagging the highest enzymatic activities, was frequently observed in stalked mucilage glands around the trap doors (see Figure 2B) and in quadrifid glands inside the traps (see Figure 2A). Other highly active structures of *Utricularia* included spherical sessile glands distributed on both trap surfaces and stalks, particularly in terrestrial species (see Figure 2D). Bifid glands in *U. vulgaris* and *U. australis* were moderately tagged for phosphatases only (see Figure 2C). Surprisingly, both substrates also tagged certain activity on various trichomes and, particularly, on the trap antennae (Table 1). Some enzymatic activity tagging the plant surfaces (*i.e.*, on traps, trichomes, stalks, etc.) was likely due to epiphytic microorganisms (probably bacteria, with characteristic single-dot tagging, *cf.* Figure 2F), some tagging pattern of antennae or rostrum was very distinct (*cf.* Figure 2E) and its epiphytic origin was unlikely. While chitinases were rarely tagged on any glands (indeed 3 of 4 such species were terrestrial), they were tagged on the antennae of all six aquatic species (*cf.* Table 1, Figure 2F). There was little difference in the phosphatase tagging of *U. vulgaris* and *U. australis* between the traps without prey and those with prey captured two days prior to labeling, whereas more outer trap structures were tagged in the traps with prey compared to those without prey of other two species (*U. floridana* and *U. intermedia*).

Commensal microorganisms, in particular algae (detected by chlorophyll *a* autofluorescence), were found in many trap fluid samples. Algal cells, however, were rarely tagged with either substrate used. Abundant *Euglena* spp. (rarely tagged for phosphatase) and other algal species occurred in the *U. vulgaris* traps (*cf.* Sirová *et al.* 2003). Another algal species (apparent "monoculture") was abundant in the *U. floridana* traps, but it was not tagged at all. One large cell of heterotrophic Eukaryote (suspect ciliate) was tagged for phosphatase in the trap fluid sample of *U. australis* and likely some suspect bacterial cell (*i.e.*, single dots of ELF alcohol fluorescence) were tagged for chitinase in the trap fluid of *U. intermedia* (*cf.* C in Table 1).

Overall, we can conclude that chitinases were almost untagged on inner trap structures and therefore could hardly participate in chitin decomposition of prey carcasses in *Utricularia* traps. On the contrary, phosphatases were much more regularly distributed on trap structures both inside and outside the traps as well as on microorganisms characterized as commensal or epiphytic. Moreover, the pattern of phosphatase tagging inside the traps with or without captured prey is largely very similar within each species suggesting that the enzyme production is constitutive, independent of prey, and supports our previous results (Sirová *et al.* 2003, unpubl.; Płachno *et al.* 2006) on the key role of phosphatases in prey digestion in *Utricularia* traps. Nevertheless, the present results together with those of Sirová *et al.* (2003) on fluorescence tagging of phosphatase activity in *Utricularia* traps are in a certain methodological controversy with the results of Płachno *et al.* (2006). The latter authors, however, applied far more concentrated ELF 97 phosphate (250 μM *vs.* 20 μM final concentrations) for tagging halved traps, as opposed to this study and Sirová *et al.* (2003) tagging intact traps. Therefore, it is obvious that the consistently very high intensity of phosphatase tagging of internal glands in halved traps in their results (Płachno *et al.* 2006; Płachno & Wołowski 2008) could be due to some penetration of the substrate into the gland cells. Nedoma *et al.* (2007) have recently proved that the 20 μM concentration of ELF 97

phosphate is adequate for tagging extracellular enzymes in plankton; also pH<8 (*i.e.*, buffering) may be important (*cf.* Štrojsová & Vrba 2006). Thus, one should be careful by applying ELF 97 substrates for tagging extracellular enzyme activities. Experimental conditions, such as substrate concentration, intactness of structures, and time of tagging, should be considered and chosen carefully.

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Reviews

Pitcher Plants of the Old World: Volume 1 (631 pages, 339 photographs); **Volume 2** (768 pages, 412 photographs). ISBN 978-0-9558918-2-3 and 978-0-9558918-3-0.
Stewart McPherson; edited by Alastair Robinson and Andreas Fleischmann.
2009. Redfern Natural History Productions, Poole, England. £34.99 each volume.

Pitcher Plants of the Old World is the result of 3 years of research on *Nepenthes* and *Cephalotus*, spending a total of 18 months in the field, and climbing over 100 mountains across Southeast Asia. This is the 4th of 5 books to date by this author dealing with carnivorous plants: Pitcher Plants of the Americas (2006), Lost Worlds of the Guiana Highlands (2008), Glistening Carnivores (2008), and the most recent, Carnivorous Plants and their Habitats (2010).

Pitcher Plants of the Old World should be considered one 1399-page treatise that is divided into two volumes containing 751 excellent photos and illustrations and 356 references.

Volume 1 contains: Introduction (9 pages), Carnivorous Plants of the World (19 p), The Pitcher Plants of the Old World (19 p), Trapping Processes (67 p), Infauna and Mutualism (27 p), *Nepenthes* (111 p), *Nepenthes* of Borneo (290 p), and *Nepenthes* of Peninsular Malaysia and Indochina (82 p).

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These two volumes cover 125 species (including 5 provisional) of *Nepenthes* plus *Cephalotus*. Each regional chapter includes a description of the area, map, table of all known species, and in-depth discussion of each species.

Although there are numerous books and articles about *Nepenthes*, this is the only publication that comprehensively deals with the genus *Nepenthes* throughout its geographical range. These volumes are highly recommended for carnivorous plant enthusiasts and professional botanists alike.
(Reviewer: Bob Ziemer)

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Mohlenbrock, R.H. 2010. Southern Comfort: Bog and pineland are blended in Georgia. Natural History (November): 34-35.

Bog areas in the southeast are described and categorized in this article. The focus is on the 650-acre Doerun Pitcherplant Bog Natural Area in southern Georgia, including its history and maintenance. Plant listings, using common names, are provided for the different habitats in the preserve. The author is a distinguished professor emeritus of botany at Southern Illinois University, Carbondale.
(Reviewer: Jim Robinson)

New Book Titles

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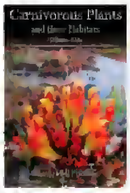

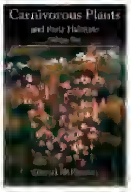

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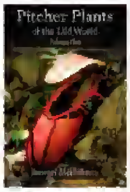

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